

Kentucky Lung Cancer Research Program

Cycle 9 Grant Abstracts

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Barbara J. Clark	UL	STARD5 Expression and Chemoresistance of Lung Adenocarcinoma Cells
Brian F. Clem	UL	Novel Small Molecule Inhibitors of Choline Kinase as a Therapeutic Strategy against Lung Cancer
Rolf Craven, Ph.D.	UK	PTEN and Akt regulation by a novel pathway in lung cancer
Howard Donninger	UL	The Role of the Novel Ras Effector, RASSF2, in Ras mediated Lung Cancer
Cicek Gercel-Taylor & Goetz Kloecker	UL	Exosomal microRNA Profiles for Differential Diagnosis for Differential Diagnosis and Disease Monitoring
Claudia Hopenhayn, Ph.D.	UK	Expanded Multifactorial Assessment of Early Stage Lung Cancer Survival in Kentucky
Venkatakrishna Jala	UL	Role of GPR30, A Novel Estrogen Receptor G-protein Coupled Receptor in the Development of Lung Cancer
Thomas L. Roszman, Ph.D.	UK	Inhibition of Lung Cancer Cell Migration/Invasion by Cell Penetrating Peptides
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Xiao-Feng Li	UL	Visualization of Hypoxia and Angiogenesis in Lung Cancer Metastasis
Craig W. Vander Kooi, Ph.D.	UK	Physical mechanisms of VEGF receptor activation and novel modes of inhibition
Zhigang Wang, Ph.D.	UK	Targeting REV1 for the prevention of lung cancer
Brian Wattenberg, Ph.D.	UL	Identification and Testing of Small Molecule Inhibitors of Sphingosine Kinase-1 as Therapeutic Agents in Lung Cancer

Principal Investigator: **Younsoo Bae, Ph.D., University of Kentucky**

Research Title: Mixed Polymer Nanoassemblies for Tunable Combination Drug Delivery to Lung Cancer Tumors

This project explores a method to achieve concurrent and sequential delivery of heat shock protein 90 (HSP90) inhibitors along with anticancer drugs to primary and metastatic lung cancer tumors, using intratumoral and intracellular pH-sensitive polymer nanoassemblies. Inhibition of HSP90 is expected to make cancers more susceptible to therapeutic agents, reducing doses and side effects while improving therapeutic efficacy. Despite the great potential, such combination cancer chemotherapy is challenging due to the limited formulation options for co-injection of multiple drugs as well as different pharmacology of various drugs in vivo. More importantly, there has been no formulation that can fix the combination settings in tumor tissues and plasma in vivo. Therefore, by completion of the proposed project, the hypothesis will be tested that: 1) mixed polymer nanoassembly drug carriers are promising to realize combination delivery of multiple drugs to the targeted tumor tissues in the body; 2) drug combination settings (e.g. concentrations, ratios and sequence of action) can be fixed in cancer cells; and 3) controlled concurrent or sequential delivery of HSP90 inhibitors and other anticancer drugs can lead to a marked increase in drug synergistic effects compared to liquid-based conventional drug formulations. Mixed polymer nanoassemblies will be prepared from biocompatible amphiphilic block copolymers comprising poly(ethylene glycol)-poly(amino acids) (PEG-PAA). Side chains of PEG-PAA will be modified with hydrazide compounds to attach drugs to the block copolymers through acidsensitive hydrazone linkages of various hydrolysis rates. A HSP90 inhibitor (geldanamycin (GDM)) and a DNA intercalator (doxorubicin (DOX)) will be concurrently conjugated to the block copolymer while their release patterns are controlled by hydrazone chemistry. Synergistic efficacy of the approach will be evaluated by calculating combination indices (CI) in order determine synergism ($CI < 0.8$), additivity ($0.8 < CI < 1.2$), and antagonism ($1.2 < CI$), using human small cell lung cancer (SCLC) cell lines (GLC4 and H82) and human non-small cell lung cancer (NSCLC) cell lines (PC-14, A549, Lu-99 and H460). Results will optimization of combination formulations for further development of mixed polymer nanoassemblies in preclinical applications.

Principal Investigator: **Barbara J. Clark, University of Louisville**

Research Title: STARD5 Expression and Chemoresistance of Lung Adenocarcinoma Cells

Principal Investigator: **Brian F. Clem, University of Louisville**

Research Title: Novel Small Molecule Inhibitors of Choline Kinase as a
Therapeutic Strategy against Lung Cancer

Principal Investigator: **Rolf Craven, Ph.D., University of Kentucky**

Research Title: PTEN and Akt regulation by a novel pathway in lung cancer

Lung cancer is responsible for 28 percent of all cancer deaths and is a particular concern in Kentucky. Traditional treatment regimens, such as chemotherapy and radiation, attack proliferating cells rather than tumor-specific protein targets, resulting in harsh side effects and limited efficacy. The development of targeted therapeutics for lung cancer represents a milestone in lung cancer treatment. One of the primary targets in lung cancer is EGFR (the epidermal growth factor receptor), a trans-membrane tyrosine kinase that drives cell proliferation. The advantage of EGFR inhibitors is that they have relatively few side effects due to the specificity of their targets in tumors. However, the disadvantage is that EGFR inhibitors have a relatively small effect on patient outcomes because tumors quickly develop resistance to them. One of the pathways associated with EGFR inhibitor resistance is directed by PTEN (phosphatase and tensin homologue on chromosome 10), a lipid/protein phosphatase that counteracts the activity of the phosphatidylinositol-3 kinase (PI3K), which activates Akt. A number of genetic studies have linked PTEN to cancer development, but the pathways regulating PTEN are poorly understood. Recently, we have joined in a collaborative effort that identified the Rak tyrosine kinase as a protein that binds and phosphorylates PTEN, stabilizing PTEN and inducing growth arrest. Rak is related to the Src family of tyrosine kinases, with SH2 and SH3 domains at its amino terminus, but Rak has a strikingly different biological activity from its relatives. Rak inhibits growth in a variety of cell types and is expressed in non-malignant lung tissue. However, Rak is not detected in several widely used lung cancer cell lines, and its expression has not been tested in lung tumors. We have found that Rak expression in A549 lung cancer cells induces growth arrest and cell death and causes a profound increase in PTEN levels. Rak is phosphorylated by Src at a key regulatory site in its carboxy terminus, and the hypothesis of the proposal is that the Rak-PTEN tumor suppressor pathway is inhibited by receptor tyrosine kinase signaling in lung cancer cells. As a result, Rak expression may be a marker of decreasing severity in lung cancer samples. We will test the hypotheses with the following specific aims. (1) Determine the extent to which EGFR de-stabilizes PTEN by suppressing Rak activation during lung tumorigenesis. (2) Test the hypothesis that Rak levels in clinical lung tumor samples predicts PTEN levels and responses to traditional therapeutic modalities. The findings will be significant because they will define a new pathway in lung cancer progression. This will ultimately provide a new therapeutic strategy for lung cancer patients, as well as a new biomarker for lung cancer progression.

Principal Investigator: **Howard Donninger, University of Louisville**

Research Title: The Role of the Novel Ras Effector, RASSF2, in Ras mediated Lung Cancer

Principal Investigator: **Cicek Gercel-Taylor, Goetz Kloecker, University of Louisville**

Research Title: Exosomal microRNA Profiles for Differential Diagnosis for Differential Diagnosis and Disease Monitoring

Principal Investigator: **Claudia Hopenhayn, Ph.D., University of Kentucky**

Research Title: Expanded Multifactorial Assessment of Early Stage Lung Cancer Survival in Kentucky

Kentucky leads the country in terms of the risk of developing or dying from lung cancer. Although survival is largely determined by how early the disease is diagnosed, there is evidence of an increasing constellation of factors that contribute to determine survival. The project proposed here will build upon our previous work to extend and expand data collection activities and add a biomarker feasibility component. Like our current study, the overall aim of this study is to describe the constellation of socioeconomic, psychological, behavioral, and geographic, as well as clinical factors that influence survival of early stage lung cancer among Kentuckians. To accomplish this, we will continue our collaboration with the Kentucky Cancer Registry (KCR) and the Kentucky Clinical Trials Network (KCTN) to collect and analyze prospectively collected data. We will also expand this study to include data on smoking behavior one year after diagnosis to assess potential change in habits following one year survival. A biomarker feasibility component will be added, to assess potential mechanisms related to the effect of smoking before and/or after diagnosis. We will use previously collected and stored samples of lymphocytes and tumor tissue for a subgroup of our study subjects, which will be obtained from the University of Kentucky Biospecimen Core Program. We will measure nucleotide excision repair (NER) capacity on cultured lymphocytes and RNA expression in normal and tumor lung tissue samples. The rationale for these biomarker assays is based on our recent findings that exposing human lung cells to cigarette smoke significantly inhibits the NER pathway, which suggests that smoking could inhibit the repair of DNA damage that is introduced by smoking, and therefore be a potential mechanism related to reduced risk of survival and/or increased risk of recurrence. This would likely increase mutations in cells of the lung formed by exposure to the DNA damaging agents present in smoke and could play a significant role in causing lung cancer.

Principal Investigator: **Venkatakrishna Jala, University of Louisville**

Research Title: Role of GPR30, A Novel Estrogen Receptor G-protein Coupled Receptor in the Development of Lung Cancer

Estrogen (17 β -estradiol, E2) is an ovarian steroid hormone essential for the growth of both normal and transformed epithelial cells during pregnancy. Estrogens are also known to promote various types of cancers including breast, endometrial cancer and ovarian cancers. Estrogen mediates its effects through two well characterized nuclear receptors, estrogen receptor α (ER α) and estrogen receptor β (ER β). There is now considerable evidence to suggest that some of the estrogen actions are independent of ER α and ER β . These include non-genomic responses such as intracellular calcium release, cAMP production, MAPK phosphorylation, *c-fos* up regulation (1-5). This prompted a search for new estrogen receptors. Recent discovery of GPR30, which suffices the membrane actions of E2 (also known as DRY12, FEG-1, LERGU, LyGPR, CMKRL2, LERGU2 and GPCR-Br) adds further complexity to estrogen biology. While many recent reports have clearly identified a role for GPR30 in E2 responsive breast cancers, its role in lung cancer remains unknown. While GPR30 is expressed normally in healthy lungs, its expression in lung tumors or in lung cancer cell lines has not been investigated. For the first time, we have observed elevated levels of GPR30 in many different lung cancer cell lines compared to immortalized normal lung cell lines. **We hypothesize that E2 signaling through GPR30 plays a critical non-redundant (independent from ER α and ER β) role in lung carcinogenesis.** Therefore, understanding the expression patterns of GPR 30 in lung cancer cells, its effect on tumorigenesis and the mechanisms of regulation would provide novel insights into the E2 action in lung tumors and blocking the GPR30 along with ERs might provide better therapeutic options for lung cancer.

Principal Investigator: **Thomas L. Roszman, Ph.D., University of Kentucky**

Research Title: Inhibition of Lung Cancer Cell Migration/Invasion by Cell Penetrating Peptides

This grant proposal examines the hypothesis that inhibition of the morphological alterations obligatory for lung cancer cell (A-549 cells) invasion and proliferation can be achieved using peptide inhibitors that are specifically designed to abrogate activation of the Ca²⁺-dependent cysteine protease, calpain II (CpII), upon which these features of malignancy are contingent. More specifically, it is proposed that blocking CpII engagement with its regulatory subunit (Rs), endoplasmic reticulum (ER) and/or Golgi apparatus (GA), will ultimately block lung cancer cell proliferation and migration (i.e. metastatic potential) and that this could have significant therapeutic value in adjunctive treatment of these tumors. To investigate this hypothesis 2 Specific Aims are proposed that will: 1) identify amino acids within the CpII protein that are required for its interactions with the Rs, ER and GA; and 2) develop a cell penetrating peptide (CPP) approach for delivering peptide inhibitors of CpII-Rs, -ER and/or -GA interactions in these cells. Accordingly, recombinant technologies will be used to mutate key amino acid domains within the CpII molecule that facilitate CpII interactions with its Rs and organelles. These mutant CpII structures subsequently will be analyzed for their impact on A-549 cell proliferation, migration and programmed cell death. Information from these studies will be used to develop peptide inhibitors of CpII activities. The efficacy of these inhibitors will be investigated using a CPP delivery approach to analyze the impact these peptides have on lung cancer cell proliferation, migration and survival. Data accrued from this study should provide insight into the role of Cp in lung cancer cell biology and provide a template for the subsequent design of in vivo models that explore the true therapeutic potential of these inhibitors.

Principal Investigator: **Woojin Lee, Ph.D., University of Kentucky**

Research Title: Immunoproteasome in lung cancer

Lung cancer is the most common cancer in the world and a highly lethal disease. The Commonwealth of Kentucky, in particular, has the highest incidence of lung cancer in the country. However, systemic treatments for lung cancer with existing chemotherapy agents are still relatively ineffective. Thus, there is an urgent need to develop novel approaches that are more effective in killing cancer cells and less toxic to non-tumor cells in order to improve the odds for lung cancer patients. In this regard, the immunoproteasome is a potential novel target for lung cancer therapy. Recently, we have developed an immunoproteasome-specific inhibitor, UK-101, and found that UK-101 induces tumor cell killing in lung and prostate cancer. The proposed study will provide crucial information on understanding how immunoproteasome inhibitor drugs kills tumor cells and also how we can predict differing responses to the immunoproteasome inhibitor drugs using specific genetic factors. This study is the first to investigate the immunoproteasome-targeting approach in solid tumor therapy and will pave the way in developing novel therapy for lung cancer.

Principal Investigator: **Xiao-Feng Li, University of Louisville**

Research Title: Visualization of Hypoxia and Angiogenesis in Lung Cancer Metastasis

Principal Investigator: **Craig W. Vander Kooi, Ph.D., University of Kentucky**

Research Title: Physical mechanisms of VEGF receptor activation and novel modes of inhibition

In order for lung cancer tumors to grow, they need new blood vessels to grow into the tumor. This process, known as tumor angiogenesis, provides vital nutrients for the tumor and is critical for the growth and progression of non-small cell lung cancer (NSCLC). Indeed, >90% of NSCLC tumors have been shown to use the key pro-angiogenic cytokine vascular endothelial growth factor (VEGF) to recruit new blood vessels. The angiogenesis inhibitor Avastin, which targets VEGF, was approved for use in 2006 in combination with carboplatin and paclitaxel as a first-line treatment for NSCLC patients. This therapy provides significant benefit, improving both time to regression and overall survival. However, the duration of effectiveness is limited and recent studies suggest that use of Avastin may actually increase tumor aggressiveness over the long term. For this reason, new targeted therapies are needed. However, there are ten known proteins involved in VEGF signaling and the details of how they function is unknown. Knowing which protein to target and how to target it is a major focus of current research. For this reason, we propose studies to first determine how the VEGF secreted by the tumor activates the two families of cell surface receptors on existing blood vessels. We then propose development and initial testing of ligand blocking peptides and activation blocking antibodies for the two families of receptors. We hypothesize that defining the common activation mechanisms coupled with design of effective inhibitors will provide unique opportunities to develop agents which compliment or replace existing angiogenesis inhibitors.

Principal Investigator: **Zhingang Wang, Ph.D., University of Kentucky**

Research Title: Targeting REV1 for the prevention of lung cancer

Lung cancer is the leading cause of cancer deaths in the United States and in the world. There is no very effective treatment for lung cancer. Therefore, novel and innovative strategies for the prevention of lung cancer are urgently needed, one of which is proposed in this application. Gene mutations are critical to the lung cancer development. In fact, gene mutations that activate the cancer-promoting genes (termed oncogenes) and destroy the anti-cancer activities of genes (termed tumor suppressor genes) have been identified. Tobacco smoking is the major cause of lung cancer, and a well-known mutagen. Gene mutation is believed to be a major mechanism of lung cancer. Although we do not know exactly how oncogenes and tumor suppressor genes are mutated in lung cells, we think that gene mutations responsible for lung cancer are most likely caused by a mechanism called the REV1-mediated mutagenesis. This gene mutation mechanism requires the REV1 gene that codes for the REV1 protein. Therefore, we believe that REV1 is absolutely important for the formation of lung cancer. We consider REV1-effected gene mutations as the foundation/building blocks for lung cancer. Therefore, we believe that lung cancer can be prevented by destroying such foundation/building blocks of cancer. Accordingly, we hypothesize that if we destroy the function of REV1 in lung, lung cancer will not be able to form and thus achieving lung cancer prevention. The human REV1 gene was originally cloned in our laboratory. Now, using a powerful genetic engineering technology called conditional gene knockout in mice, we have created Rev1-conditional knockout mice. The REV1 function in the lung of these transgenic mice can be destroyed in the laboratory like a laser-guided missile aiming at the Rev1 target with pinpoint accuracy. Using this mouse system, we can now test the feasibility of our novel strategy in lung cancer prevention. If lung cancer in mice is abolished or largely diminished after the REV1 function is destroyed, the results will be highly significant in two ways: (a) we will have known exactly how anti-cancer genes and cancer-causing genes are mutated in lung cells; and (b) we will have proven that the REV1 protein is indeed a highly valuable drug target for the prevention of lung cancer. Accomplishing this project will allow us to develop REV1 inhibitors as therapeutic drugs for the prevention of lung cancer in subsequent studies. We envision that someday in the future such a drug may be used as a nutritional supplement by people to greatly reduce the incidence or risk of lung cancer. Preventing lung cancer by inhibiting REV1 is a potentially revolutionary strategy in our battle against lung cancer. If lung cancer can be prevented by inhibiting REV1, the contribution to human health will be enormous.

Principal Investigator: **Brian Wattenberg, Ph.D., University of Louisville**

Research Title: Identification and Testing of Small Molecule Inhibitors of Sphingosine Kinase-1 as Therapeutic Agents in Lung Cancer

The goal of the research proposed here is to develop novel, improved inhibitors of the signaling enzyme sphingosine kinase as potential anti-cancer therapeutics. Sphingosine kinase is a signaling enzyme that produces the potent pro-proliferative second messenger sphingosine-1-phosphate. Sphingosine kinase has recently emerged as a therapeutic target in the treatment of cancer. Sphingosine kinase is substantially upregulated in a variety of human cancers and promotes cell growth, blocks apoptosis, and produces a tumorigenic phenotype when overexpressed in cells in culture. Moreover inhibitors of sphingosine kinase inhibit tumor growth in animal models. Here, we first aim to discover inhibitors which block the activation of sphingosine kinase. Secondly we will embark on the crystallization of sphingosine kinase, which has yet to be accomplished, to enable discovery and design of enzyme inhibitors. Sphingosine kinase is activated by the MAP kinase Erk2. Here we will define the docking site between these two proteins and utilize this knowledge to produce inhibitors of the docking interaction and thus the activation of sphingosine kinase. The three-dimensional structure of sphingosine kinase (and all homologous proteins) is unknown because of difficulties in producing diffractable crystals due to the instability of the protein. Here we propose a strategy to use advanced technologies to identify mutations that stabilize sphingosine kinase and enable its crystallization. This will provide the basis for future work, beyond the scope of this proposal, to determine the three-dimensional structure of sphingosine kinase.